STATE OF MISSOURI **DEPARTMENT OF NATURAL RESOURCES**

MISSOURI CLEAN WATER COMMISSION



MISSOURI STATE OPERATING PERMIT

In compliance with the Missouri Clean Water Law, (Chapter 644 R.S. Mo. as amended, hereinafter, the Law), and the Federal Water Pollution Control Act (Public Law 92-500, 92nd Congress) as amended,

MO-0097837

Permit No.:

Owner: Address:	City of Columbia PO Box 6015, Columbia, MO 65205
Continuing Authority: Address:	Same as above Same as above
Facility Name: Address:	Columbia Regional Wastewater Treatment Plant 4900 West Gillespie Bridge Road, Columbia, MO 65203
Legal Description:	See page 2
Receiving Stream: First Classified Stream and ID: USGS Basin & Sub-watershed No.: is authorized to discharge from the facil as set forth herein: FACILITY DESCRIPTION	See page 2 See page 2 See page 2 lity described herein, in accordance with the effluent limitations and monitoring requirements
See page 2	
	discharges under the Missouri Clean Water Law and the National Pollutant Discharge other regulated areas. This permit may be appealed in accordance with Section 644.051.6 of
July 29, 2005 Effective Date	Doyle Childers, Director, Department of Natural Resources Executive Secretary, Clean Water Commission
July 28, 2010 Expiration Date MO 780-0041 (10-93)	Edward Galbraith, Director of Staff, Clean Water Commission

FACILITY DESCRIPTION (continued)

<u>Outfall #001</u> – POTW – SIC #4952

Activated sludge/anaerobic sludge digester/sludge is being land applied/sludge storage basin/wetland treatment.

Design population equivalent is 174,058.

Design flow is 20.6 MGD. Actual flow is 15.5 MGD.

Design sludge production is 3,948 dry tons/year. Actual sludge production is 3,787 dry tons/year.

Legal Description: Sec. 18, T47N, R13W, Boone County Receiving Stream: Eagle Bluffs Conservation Area (U)

First Classified Stream & ID: Missouri River (P) (00701) USGS Basin and Subwatershed: (10300102–110008)

Outfall #002 - POTW - SIC #4952

Emergency Discharge only, due to inability to get wastewater to the wetlands (flooding, line break or blockage, construction, etc.)

Legal Description: NE ¼, SW ¼, Sec. 29, T48N, R13W, Boone County

Receiving Stream: Hinkson Creek (P)

First Classified Stream & ID: Hinkson Creek (P) (01007) USGS Basin and Subwatershed: (10310102–120002)

Outfall #003 - POTW - SIC #4952

Stormwater discharge from wastewater plant grounds.

Flow dependent upon precipitation.

Legal Description: NE ¼, SW ¼, Sec. 29, T48N, R13W, Boone County

Receiving Stream: Hinkson Creek (P)
First Classified Stream & ID: Hinkson Creek (P) (01007)
USGS Basin & Subwatershed: (10300102–120002)

Outfall #004 – POTW – SIC #4952

Stormwater discharge from wastewater plant grounds.

Flow dependent upon precipitation.

Legal Description: SE 1/4, NW 1/4, Sec. 29, T48N, R13W, Boone County

Receiving Stream: Unnamed Tributary to Hinkson Creek (U)

First Classified Stream & ID: Hinkson Creek (P) (01007) USGS Basin & Subwatershed: (10300102-120002)

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PERMIT NUMBER MO-0097837

A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:

		FINAL EFFLUENT LIMITATIONS		MONITORING REQUIREMENTS			
OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE	
Outfall #001 Flow	MGD	*		*	once/weekday**	24 hr. total	
Biochemical Oxygen Demand ₅ ***	mg/L		45	30	once/weekday**	24 hr. composite	
Total Suspended Solids*** (Note 1)	mg/L		45	30	once/weekday**	24 hr. composite	
pH – Units	SU	****		****	once/weekday**	grab	
MONITORING REPORTS SHALL BE SUBMIT	TTED <u>MONT</u>	HLY; THE F	IRST REPOR	T IS DUE <u>s</u>	eptember 28, 2005		
Whole Effluent Toxicity (WET) Test %	Survival	See S	Special Cond	itions	once/year	24 hr. composite	
MONITORING REPORTS SHALL BE SUBMI	ΓΤΕD <u>ANNU</u>	ALLY; THE	FIRST REPO	RT IS DUE _	October 28, 2006 .		
Arsenic, Total Recoverable	μg/L	160			once/quarter****	24 hr. composite	
Copper,Total Recoverable	μg/L	464			once/quarter****	24 hr. composite	
Zinc,Total Recoverable	μg/L	*			once/quarter****	24 hr. composite	
Nickel,Total Recoverable	μg/L	*			once/quarter****	24 hr. composite	
Cadmium,Total Recoverable	μg/L	576			once/quarter****	24 hr. composite	
Mercury,Total Recoverable	μg/L	19			once/quarter****	24 hr. composite	
Lead,Total Recoverable	μg/L	1520			once/quarter****	24 hr. composite	
Chromium, Total Recoverable	μg/L	496			once/quarter****	24 hr. composite	
Silver,Total Recoverable	μg/L	104			once/quarter****	24 hr. composite	
Cyanide, Total Recoverable	μg/L	176			once/quarter****	grab	
MONITORING REPORTS SHALL BE SUBMITTED QUARTERLY; THE FIRST REPORT IS DUE _October 28, 2005							
Outfall #002 – Emergency Discharge							
Flow	MGD		*		once/day	24 hr. total	
Biochemical Oxygen Demand	mg/L		45		once/day	grab	
Total Suspended Solids	mg/L		45		once/day	grab	
pH – Units	SU		****		once/day	grab	
Ammonia as N	mg/L		*		once/day	grab	

MONITORING REPORTS SHALL BE SUBMITTED MONTHLY; THE FIRST REPORT IS DUE <u>September 28, 2005</u>. THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.

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PERMIT NUMBER MO-0097837

MONITORING REQUIREMENTS

A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:

FINAL EFFLUENT LIMITATIONS

OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE
Outfalls #003 & 004 (Note 2)					Ç 2	
Flow	MGD		*	*	once/quarter****	grab
Biochemical Oxygen Demand	mg/L		*	*	once/quarter****	grab
Total Suspended Solids	mg/L		*	*	once/quarter****	grab
pH - Units	SU		*	*	once/quarter****	grab
MONITORING REPORTS SHALL BE SUBMITTED QUARTERLY; THE FIRST REPORT IS DUE October 28, 2005.						
Sludge Lagoon						
Lagoon Freeboard (Note 3)	feet	*			once/quarter****	depth measurement
Groundwater Monitoring Wells at On- Site Sl	udge Applica	ation Area (N	Note 4)			
Water Level	feet	*			once/month	depth measurement
Total Suspended Solids	mg/L	*			once/month	grab
Total Dissolved Solids*****	mg/L	*			once/month	grab
Nitrate Nitrogen as N	mg/L	10			once/month	grab
Ammonia Nitrogen as N	mg/L	*			once/month	grab
pH - Units	SU	****		****	once/month	grab
Copper,Total Recoverable	μg/L	1000			once/year	grab
Zinc,Total Recoverable	μg/L	5000			once/year	grab
Nickel,Total Recoverable	μg/L	100			once/year	grab
Cadmium, Total Recoverable	μg/L	5			once/year	grab
Mercury, Total Recoverable	μg/L	2			once/year	grab
Lead, Total Recoverable	μg/L	15			once/year	grab
Silver,Total Recoverable	μg/L	50			once/year	grab
Arsenic, Total Recoverable	μg/L	50			once/year	grab
Manganese, Total Recoverable	μg/L	*			once/year	grab
Cyanide, Total Recoverable	μg/L	50			once/year	grab
Molybdenum, Total Recoverable	μg/L	*			once/year	grab
Selenium, Total Recoverable	μg/L	10			once/year	grab
Aluminum,Total Recoverable MONITORING REPORTS SHALL BE SUBMIT	μg/L	*	TIDGE PERCE	THE PLEE	once/year	grab E SHALL BE NO

MONITORING REPORTS SHALL BE SUBMITTED <u>ANNUALLY</u>; THE FIRST REPORT IS DUE <u>October 28, 2006</u>. THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.

B. STANDARD CONDITIONS

IN ADDITION TO SPECIFIED CONDITIONS STATED HEREIN, THIS PERMIT IS SUBJECT TO THE ATTACHED <u>Parts I, II & III</u> STANDARD CONDITIONS DATED <u>October 1, 1980 and August 15, 1994</u>, AND HEREBY INCORPORATED AS THOUGH FULLY SET FORTH HEREIN.

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PERMIT NUMBER MO-0097837

A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:

	FINAL EFFLUENT LIMITATIONS			MONITORING REQUIREMENTS			
OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE	
Groundwater Monitoring at Wetland Cells Area (Note 5)							
Water Level	feet	*			once/quarter****	depth	
Conductivity	umhos/cm	*			once/quarter****	measurement grab	
Nitrate Nitrogen	mg/L	*			once/quarter****	grab	
Total Nitrogen	mg/L	*			once/quarter****	grab	
Copper, Total Recoverable	μg/L	*			once/quarter****	grab	
Zinc,Total Recoverable	μg/L	*			once/quarter****	grab	
Nickel, Total Recoverable	μg/L	*			once/quarter****	grab	
Cadmium, Total Recoverable	μg/L	*			once/quarter****	grab	
Mercury, Total Recoverable	μg/L	*			once/quarter****	grab	
Lead, Total Recoverable	μg/L	*			once/quarter****	grab	
Silver, Toal Recoverable	μg/L	*			once/quarter****	grab	
Manganese, Total Recoverable	μg/L	*			once/quarter****	grab	
pH– Units	SU	****		****	once/quarter****	grab	

MONITORING REPORTS SHALL BE SUBMITTED <u>ANNUALLY</u>; THE FIRST REPORT IS DUE <u>October 28, 2006</u>. THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.

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MO 780-0010 (8/91)

A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS (continued)

- * Monitoring requirement only.
- ** Once each weekday means: Monday, Tuesday, Wednesday, Thursday, and Friday.
- *** This facility is required to meet a removal efficiency of 85% or more.
- **** pH is measured in pH units and is not to be averaged. The pH is limited to the range of 6.0-9.0 pH units.
- ***** Once per quarter in the months of March, June, September, and November.
- ***** An estimated TDS value calculated from conductivity is acceptable.

Note 1- Total Suspended Solids (TSS) may be exceeded periodically due to heavy use by waterfowl. The effluent limits for TSS during these periods shall be a weekly average of 100 mg/L and monthly average of 70 mg/L. Each instance of heavy waterfowl usage shall be documented by the City and confirmed by the Missouri Department of Conservation.

Note 2 – Monitoring will only be required at the request of the department due to the city's implementation of it's Stormwater Pollution Prevention Plan prepared by Joel Gambill on March 19, 2003.

Note 3 – Depth measured from the top of the supernatant overflow weir to the top of the sludge layer.

A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS (continued)

Note 4 – Ground water samples will be collected from the monitoring wells located on the dedicated sludge disposal site. The depth of the water in the well shall be measured from the top of the well casing.

Note 5 - Ground water samples will be collected from monitoring wells located around the Wetland Area (eight locations, two wells per location). These include MW1-1A & MW1-1B, MW1-2A & MW1-2B, MW1-3A & MW1-3B, MW1-4A & MW1-4B, MW2-1A & MW2-1B, MW3-1A & MW3-1B, MW4-1A & MW4-1B, and MW4-2A & MW4-2B. Wells MW2-2A and MW2-2B will no longer be sampled. The depth of water in the well shall be measured from the top of the wall casing. The sample may be collected from the shallowest well at each location which contains ground water.

C. SPECIAL CONDITIONS

- 1. This permit may be reopened and modified, or alternatively revoked and reissued, to:
 - (a) Comply with any applicable effluent standard or limitation issued or approved under Sections 301(b)(2)(C) and (D), 304(b)(2), and 307(a) (2) of the Clean Water Act, if the effluent standard or limitation so issued or approved:
 - (1) contains different conditions or is otherwise more stringent than any effluent limitation in the permit; or
 - (2) controls any pollutant not limited in the permit.
 - (b) Incorporate new or modified effluent limitations or other conditions, if the result of a waste load allocation study, toxicity test or other information indicates changes are necessary to assure compliance with Missouri's Water Quality Standards.
 - (c) ©Incorporate new or modified effluent limitations or other conditions if, as the result of a watershed analysis, a Total Maximum Daily Load (TMDL) limitation is developed for the receiving waters which are currently included in Missouri's list of waters of the state not fully achieving the state's water quality standards, also called the 303(d) list.

The permit as modified or reissued under this paragraph shall also contain any other requirements of the Clean Water Act then applicable.

- 2. All outfalls must be clearly marked in the field.
- 3. Changes in Discharges of Toxic Substances

The permittee shall notify the Director as soon as it knows or has reason to believe:

- (a) That any activity has occurred or will occur which would result in the discharge of any toxic pollutant which is not limited in the permit, if that discharge will exceed the highest of the following "notification levels:"
 - (1) One hundred micrograms per liter (100 μ g/L);
 - (2) Two hundred micrograms per liter (200 μ g/L) for acrolein and acrylonitrile; five hundred micrograms per liter (500 μ g/L) for 2,5 dinitrophenol and for 2-methyl-4, 6-dinitrophenol; and one milligram per liter (1 mg/L) for antimony;
 - (3) Five (5) times the maximum concentration value reported for the pollutant in the permit application;
 - (4) The level established in Part A of the permit by the Director.
- (b) That they have begun or expect to begin to use or manufacture as an intermediate or final product or byproduct any toxic pollutant, which was not reported in the permit application.
- 4. Report as no-discharge when a discharge does not occur during the report period.

C. SPECIAL CONDITIONS (continued)

5. Water Quality Standards

- (a) Discharges to waters of the state shall not cause a violation of water quality standards rule under 10 CSR 20-7.031, including both specific and general criteria.
- (b) General Criteria. The following general water quality criteria shall be applicable to all waters of the state at all times including mixing zones. No water contaminant, by itself or in combination with other substances, shall prevent the waters of the state from meeting the following conditions:
 - (1) Waters shall be free from substances in sufficient amounts to cause the formation of putrescent, unsightly or harmful bottom deposits or prevent full maintenance of beneficial uses;
 - (2) Waters shall be free from oil, scum and floating debris in sufficient amounts to be unsightly or prevent full maintenance of beneficial uses;
 - (3) Waters shall be free from substances in sufficient amounts to cause unsightly color or turbidity, offensive odor or prevent full maintenance of beneficial uses;
 - (4) Waters shall be free from substances or conditions in sufficient amounts to result in toxicity to human, animal or aquatic life;
 - (5) There shall be no significant human health hazard from incidental contact with the water;
 - (6) There shall be no acute toxicity to livestock or wildlife watering;
 - (7) Waters shall be free from physical, chemical or hydrologic changes that would impair the natural biological community;
 - (8) Waters shall be free from used tires, car bodies, appliances, demolition debris, used vehicles or equipment and solid waste as defined in Missouri's Solid Waste Law, section 260.200, RSMo, except as the use of such materials is specifically permitted pursuant to section 260.200-260.247.
- 6. Sludge and Biosolids Use For Domestic Wastewater Treatment Facilities
 - (a) Permittee shall comply with the pollutant limitations, monitoring, reporting, and other requirements in accordance with the attached permit Standard Conditions.
 - (b) If sludge is not removed by a contract hauler, permittee is authorized to land apply biosolids. Permit Standard Conditions, Part III shall apply to the land application of biosolids. Permittee shall notify the department at least 180 days prior to the planned removal of biosolids. The department may require submittal of a biosolids management plan for department review and approval as determined appropriate on a case-by-case basis.
- 7. Whole Effluent Toxicity (WET) tests will be conducted as follows:

SUMMARY OF WET TESTING FOR THIS PERMIT							
OUTFALL	A.E.C. %	FREQUENCY	SAMPLE TYPE	MONTH			
#001	100%	Annually	24 hr. composite	August			

- (a) Test Schedule and Follow-Up Requirements
 - (1) Perform a single-dilution test in the months and at the frequency specified above. If the effluent passes the test, do not repeat the test until the next test period.
 Submit test results along with complete copies of the test reports as received from the laboratory within 30 calendar days of availability to the WPCP, Water Quality Section, P.O. Box 176, Jefferson City, MO 65102.

C. SPECIAL CONDITIONS (continued)

- 7. Whole Effluent Toxicity (WET) (continued)
 - (a) Test Schedule and Follow-Up Requirements (continued)
 - (2) If the effluent fails the test, a multiple dilution test shall be performed within 30 calendar days, and biweekly thereafter, until one of the following conditions are met:
 - (a) THREE CONSECUTIVE MULTIPLE-DILUTION TESTS PASS. No further tests need to be performed until next regularly scheduled test period.
 - (b) A TOTAL OF THREE MULTIPLE-DILUTION TESTS FAIL.
 - (3) The permittee shall submit a summary of all test results for the test series along with complete copies of the test reports as received from the laboratory to the WPCP, Planning Section, P.O. Box 176, Jefferson City, MO 65102 within 14 calendar days of the third failed test.
 - (4) Additionally, the following shall apply upon failure of the third test: A toxicity identification evaluation (TIE) or toxicity reduction evaluation (TRE) is automatically triggered. The permitee shall contact WPCP, Planning Section to ascertain as to whether a TIE or TRE is appropriate. The permittee shall submit a plan for conducting a TIE or TRE to the Planning Section of the WPCP within 60 calendar days of the date of DNR's direction to perform either a TIE or TRE. This plan must be approved by DNR before the TIE or TRE is begun. A schedule for completing the TIE or TRE shall be established in the plan approval.
 - (5) Upon DNR's approval, the TIE/TRE schedule may be modified if toxicity is intermittent during the TIE/TRE investigations. A revised WET test schedule may be established by DNR for this period.
 - (6) If a previously completed TIE has clearly identified the cause of toxicity, additional TIEs will not be required as long as effluent characteristics remain essentially unchanged and the permittee is proceeding according to a DNR approved schedule to complete a TRE and reduce toxicity. Regularly scheduled WET testing as required in the permit, without the follow-up requirements, will be required during this period.
 - (7) All failing test results shall be reported to WPCP, Planning Section, P.O. Box 176, Jefferson City, MO 65102within 14 calendar days of the availability of the results.
 - (8) When WET test sampling is required to run over one DMR period, each DMR report shall contain information generated during the reporting period.
 - (9) Submit a concise summary of all test results with the annual report.
 - (b) PASS/FAIL procedure and effluent limitations:
 - (1) To pass a single-dilution test, mortality observed in the AEC test concentration shall not be significantly different (at the 95% confidence level; p = 0.05) than that observed in the upstream receiving-water control sample. The appropriate statistical tests of significance will be those outlined in the most current USEPA acute toxicity manual or those specified by the MDNR.
 - (2) To pass a multiple-dilution test:
 - (a) the computed percent effluent at the edge of the zone of initial dilution, Acceptable Effluent Concentration (AEC), must be less than three-tenths (0.3) of the LC₅₀ concentration for the most sensitive of the test organisms; or,
 - (b) all dilutions equal to or greater than the AEC must be nontoxic. Failure of one multiple-dilution test is an effluent limit violation.

C. SPECIAL CONDITIONS (continued)

- 7. Whole Effluent Toxicity (WET) (continued)
 - (c) Test Conditions
 - (1) Test Type: Acute Static non-renewal
 - (2) Test species: Ceriodaphnia dubia and Pimephales promelas (fathead minnow). Organisms used in WET testing shall come from cultures reared for the purpose of conducting toxicity tests and cultured in a manner consistent with the most current USEPA guidelines. All test animals shall be cultured as described in the most current edition of Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms.
 - (3) Test period: 48 hours at the "Acceptable Effluent Concentration" (AEC) specified above.
 - (4) When dilutions are required, upstream receiving stream water shall be used as dilution water. If upstream water is unavailable or if mortality in the upstream water exceeds 10%, "reconstituted" water will be used as dilution water. Procedures for generating reconstituted water will be supplied by the MDNR upon request.
 - (5) Single-dilution tests will be run with:
 - (a) Effluent at the AEC concentration;
 - (b) 100% receiving-stream water (if available), collected upstream of the outfall at a point beyond any influence of the effluent; and
 - (c) reconstituted water.
 - (6) Multiple-dilution tests will be run with:
 - (a) 100%, 50%, 25%, 12.5%, and 6.25% effluent, unless the AEC is less than 25% effluent, in which case dilutions will be 4 times the AEC, two times the AEC, AEC, ½ AEC and ¼ AEC;
 - (b) 100% receiving-stream water (if available), collected upstream of the outfall at a point beyond any influence of the effluent; and
 - (c) reconstituted water.
 - (7) If reconstituted-water control mortality for a test species exceeds 10%, the entire test will be rerun.

SUMMARY OF TEST METHODOLOGY FOR WHOLE-EFFLUENT TOXICITY TESTS

Whole-effluent-toxicity test required in NPDES permits shall use the following test conditions when performing single or multiple dilution methods. Any future changes in methodology will be supplied to the permittee by the Missouri Department of Natural Resources (MDNR). Unless more stringent methods are specified by the DNR, the procedures shall be consistent with the most current edition of Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms,

Test conditions for Ceriodaphnia dubia:

Test duration: 48 l

Temperature: $25 \pm 1^{\circ}\text{C}$ Temperatures shall not deviate by more than 3°C

during the test.

Light Quality: Ambient laboratory illumination

Photoperiod: 16 h light, 8 h dark Size of test vessel: 30 mL (minimum) Volume of test solution: 15 mL (minimum)

Age of test organisms: <24 h old

No. of animals/test vessel: 5
No. of replicates/concentration: 4

No. of organisms/concentration: 20 (minimum)

Feeding regime: None (feed prior to test)

Aeration: None

Dilution water: Upstream receiving water; if no upstream flow, synthetic

water modified to reflect effluent hardness.

Endpoint: Pass/Fail (Statistically significant Mortality when compared

to upstream receiving water control or synthetic control if

upstream water was not available at p < 0.05)

Test acceptability criterion: 90% or greater survival in controls

Test conditions for (Pimephales promelas):

No. of organisms/concentration:

Test duration: 48 h

Temperature: $25 \pm 1^{\circ}\text{C}$ Temperatures shall not deviate by more than 3°C

during the test.

Light Quality: Ambient laboratory illumination

Photoperiod: 16 h light/ 8 h dark
Size of test vessel: 250 mL (minimum)
Volume of test solution: 200 mL (minimum)
Age of test organisms: 1-14 days (all same age)

No. of animals/test vessel:

No. of replicates/concentration: 4 (minimum) single dilution method

2 (minimum) multiple dilution method 40 (minimum) single dilution method

Feeding regime: 20 (minimum) multiple dilution method None (feed prior to test)

Aeration: None, unless DO concentration falls below 4.0 mg/L; rate

should not exceed 100 bubbles/min.

Dilution water: Upstream receiving water; if no upstream flow, synthetic

water modified to reflect effluent hardness.

Endpoint: Pass/Fail (Statistically significant Mortality when compared

to upstream receiving water control or synthetic control if

upstream water was not available at p < 0.05)

Test Acceptability criterion: 90% or greater survival in controls